98 Rec'd PCT/PTO 01 FEB 2002

FORM PTO-1390 (REV 5-93)	U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY DOCKET NO. 100564-00097
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		DATE: February 1, 2002
		U.S. APPLN. NO. (IF KNOWN, SEE 37 C.F.R. 1.5) No. 10/030182
INTERNATIONAL APPLICATION NO. PCT/EP00/07445	INTERNATIONAL FILING DATE August 1, 2000	PRIORITY DATE CLAIMED August 1, 1999

TITLE OF INVENTION: MICROFLUIDIC REACTION SUPPORT HAVING THREE FLOW LEVELS AND A TRANSPARENT COVER LAYER

APPLICANT(S) FOR DO/EO/US: Cord Friedrich STAHLER, Manfred MULLER and Peer Friedrich STAHLER

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
(THE BASIC FILING FEE IS ATTACHED)

2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.

3. ☒ This express request to begin national examination procedures [35 U.S.C. 371(f)] at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).

4. ☒ A proper demand for International Preliminary Amendment was made by the 19th month from the earliest claimed priority date.

5. ☒ A copy of the International Application as filed [35 U.S.C. 371(c)(2)]
   a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).

6. ☒ A translation of the International Application into English [35 U.S.C. 371(c)(2)].

7. ☒ Amendments to the claims of the International Application under PCT Article 19 [35 U.S.C. 371(c)(3)]
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.

8. ☐ A translation of the amendments to the claims under PCT Article 19 [35 U.S.C. 371(c)(3)].

9. ☐ An oath or declaration of the inventor(s) [35 U.S.C. 371(c)(4)].

10. ☒ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 [35 U.S.C. 371(c)(5)].

Items 11 - 16 below concern other document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 C.F.R. 1.97 and 1.98.

12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 C.F.R. 3.28 and 3.31 is included.

13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.

14. ☐ A substitute specification.

15. ☐ A change of power of attorney and/or address letter.

16. ☐ Other items or information: PCT/ISA/210 in German & English; PCT/IPEA/416; PCT/RO/101
Drawings (19 sheets, Figs 1a-20)

JC03 Rec'd PCT/PTO 01 FEB 2002

U.S. APPLN. NO. (IF KNOWN SEE 37 C.F.R. 1.50) NEW 10/030182	INTERNATIONAL APPLICATION NO. PCT/EP00/07445	ATTORNEY DOCKET NO. 100564-00097 DATE: February 1, 2002

17. ☒ The following fees are submitted:
Basic National Fee [37 C.F.R. 1.492(a)(1)-(5)]:
Search Report has been prepared by the EPO or JPO............$890.00
International preliminary examination fee paid to USPTO (37 C.F.R. 1.482)..$710.00
No international preliminary examination fee paid to USPTO (37 C.F.R. 1.482) but international search fee paid to USPTO [37 C.F.R. 1.445(a)(2)].....................................$740.00
Neither international preliminary examination fee (37 C.F.R. 1.482) or international search fee [37 C.F.R. 1.445(a)(2)] paid to USPTO......................$1,040.00
International preliminary examination fee paid to USPTO (37 C.F.R. 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4)..$ 100.00

	CALCULATIONS	PTO USE ONLY
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 890.00	
Surcharge of $130.00 for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date [37 C.F.R. 1.492(e)].	$	

Claims	Number Filed	Number Extra	Rate		
Total Claims	32 - 20 =	12	X $ 18.00	$ 216.00	
Independent Claims	3 - 3 =	0	X $ 84.00	$	
Multiple dependent claim(s) (if applicable)			+ $280.00	$	

TOTAL OF ABOVE CALCULATIONS =	$ 1,106.00	
Reduction by one-half for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 C.F.R. 1.9, 1.27, 1.28).	$	
SUBTOTAL =	$ 1,106.00	
Processing fee of $130.00 for furnishing the English translation later the ☐ 20 ☐ 30 months from the earliest claimed priority date [37 C.F.R. 1.492(f)]. +	$	
TOTAL NATIONAL FEE =	$ 1,106.00	
Fee for recording the enclosed assignment [37 C.F.R. 1.21(h)]. The assignment must be accompanied by an appropriate cover sheet (37 C.F.R. 3.28, 3.31). $40.00 per property +	$	
TOTAL FEES ENCLOSED =	$ 1,106.00	
	Amount to be refunded	$
	Charged	$

a. ☒ A check in the amount of $1,106.00 to cover the above fees is enclosed.
b. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 01-2300.

NOTE: Where an appropriate time limit under 37 C.F.R. 1.494 or 1.495 has not been met, a petition to revive [37 C.F.R. 1.137(a) or (b)] must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:
Arent Fox Kintner Plotkin & Kahn
1050 Connecticut Avenue, N.W.
Suite 400
Washington, D.C. 20036-5339
Tel: (202) 857-6000 Fax: (202) 638-4810
RBM/epb

Robert B. Murray
Reg. No. 22,980

10/030182

JC03 Rec'd PCT/PTO 01 FEB 2002

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of: Cord F. STAHLER et al.

Appln. No.: PCT/EP00/07445

Filed: Concurrently herewith   Attorney Dkt. No.: 100564-00097

For: MICROFLUIDIC REACTION SUPPORT HAVING THREE FLOW LEVELS AND A TRANSPARENT COVER LAYER

PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, D.C. 20231   Date: February 1, 2002

Sir:

Prior to calculation of the filing fees and initial examination of the application, please amend the above-identified application as follows:

IN THE SPECIFICATION:

Before Line 1, page 1 insert

--CROSS-REFERENCE TO RELATED APPLICATION

This application is a National Stage entry of International Application No. PCT/EP00/07445, filed August 1, 2000, the entire specification claims and drawings of which are incorporated herewith by reference. --

IN THE CLAIMS:

Please amend claims 4-10, 1, 14, 16-20, 22, 23, 25-28 and 31-32 as follows:

4. (Amended) The use of a microfluidic reaction support as claimed in claim 1, in which each flow channel (2, 3; 102, 103; 202, 203) can individually be charged with fluid and discharged via a valve system.

5. (Amended) The use of a microfluidic reaction support as claimed in claim 1, in which the flow channel structure is provided on one side or on both sides with a transparent cover layer.

6. (Amended) The use of a microfluidic reaction support as claimed in claim 1 for wet-chemical synthesis of oligomeric or polymeric probes such as DNA, RNA, PNA, LNA, and others.

7. (Amended) The use of a microfluidic reaction support as claimed in claim 1 for integrated synthesis and analysis of polymers.

8. (Amended) The use of a microfluidic reaction support as claimed in claim 1 for optical analysis of the hybridization of synthesized polymeric probes with complementary fragments.

9. (Amended) The use of a microfluidic reaction support as claimed in claim 1 for efficient highly parallel combined wet-chemical and light-controlled synthesis of oligomeric or polymeric probes such as DNA, RNA, PNA, LNA, proteins and also for subsequent optical analysis of the hybridization with complementary fragments.

10. (Amended) The use of a microfluidic reaction support as claimed in claim 1 for light-controlled synthesis of oligomeric or polymeric probes such as DNA, RNA, PNA, LNA and also for subsequent optical analysis of the hybridization with complementary fragments.

13. (Amended) The use of a microfluidic reaction support as claimed in claim 1, in which the walls between the feed channels (2; 102; 202) and the discharge channels (3; 103; 203) are made lightproof.

14. (Amended) The use of a microfluidic reaction support as claimed in claim 2, in which the connecting channels (104; 204) consist of a multiplicity of glass fiber bundles fused together from which the glass fiber cores have been etched out, thus resulting in microchannels.

16. (Amended) The use of a microfluidic reaction support as claimed in claim 2, in which the levels consist of a silicon layer into which a multiplicity of small channels has been etched.

17. (Amended) The use of a microfluidic reaction support as claimed in claim 2, in which a plurality of levels with flow channels are arranged on top of one another such that the reaction areas in the projection perpendicular to the flow levels are not superimposed and can be photoactivated individually by light and light can be detected, likewise location-specifically, for each of the reaction areas.

18. (Amended) The use of microfluidic reaction support as claimed in claim 1, in which a programmable light source matrix is integrated into the reaction support.

19. (Amended) The use of a microfluidic reaction support as claimed in claim 1, in which a detection unit in the form of a CCD matrix is integrated into the reaction support.

20. (Amended) The use of a microfluidic reaction support as claimed in claim 1, in which a multiplicity of in each case different receptors is bound to specific areas to the support.

22. The use of a microfluidic reaction support as claimed in claim 20, in which the receptors have been synthesized on the support from individual synthesis building blocks.

23. (Amended) The use of a microfluidic reaction support as claimed in claim 20, in which a building block is introduced between receptor and support, which allows the receptor to be removed by cleavage.

25. (Amended) The use of a microfluidic reaction support as claimed in claim 5, in which luminescence and fluorescence are measured through the transparent cover layer in a backlight process.

26. (Amended) The use of a microfluidic reaction support as claimed in claim 5 with covering on both sides by transparent cover layers, in which each reaction area is exposed to light of a defined wavelength via a programmable light source matrix and is biochemically functionalized via the light and the supply of fluid and all processes in the reaction support are optically monitored simultaneously via the second transparent cover layer.

27. (Amended) The use of a microfluidic reaction support as claimed in claim 5 with covering on both sides by transparent cover layers, in which luminescence and fluorescence and also absorption are measured through the two transparent cover layers in a transmitted-light process.

28. (Amended) The use of a microfluidic reaction support as claimed in claim 1 for individual wetting and biochemical functionalization of each reaction area in the reaction support.

31. (Amended) The microfluidic reaction as claimed in claim 29, in which the fluid feed channels (202) cross the fluid discharge channels (203) at an angle in a projection perpendicular to the first and second level.

32. (Amended) The microfluidic reaction support as claimed in claim 29, in which the fluid discharge channels (102) run parallel to the fluid feed channels (103).

REMARKS

Claims 1-32 are pending in this application. By this Amendment, claims 4-10, 13, 14, 16-20, 22, 23, 25-28, 31 and 32 are amended to correct delete multiple dependency thereof and to place this application into better condition for examination. No new matter is added.

Respectfully submitted,

Robert B. Murray
Registration No. 22,980

ARENT FOX KINTNER PLOTKIN & KAHN, PLLC
1050 Connecticut Avenue, N.W.,
Suite 400
Washington, D.C. 20036-5339
Tel: (202) 857-6000
Fax: (202) 638-4810
RBM/epb

Marked-up Claims

4. (Amended) The use of a microfluidic reaction support as claimed in [any of the preceding] claim[s] 1, in which each flow channel (2, 3; 102, 103; 202, 203) can individually be charged with fluid and discharged via a valve system.

5. (Amended) The use of a microfluidic reaction support as claimed in [any of the preceding] claim[s] 1, in which the flow channel structure is provided on one side or on both sides with a transparent cover layer.

6. (Amended) The use of a microfluidic reaction support as claimed in [any of the preceding] claim[s] 1 for wet-chemical synthesis of oligomeric or polymeric probes such as DNA, RNA, PNA, LNA, and others.

7. (Amended) The use of a microfluidic reaction support as claimed in [any of the preceding] claim[s] 1 for integrated synthesis and analysis of polymers.

8. (Amended) The use of a microfluidic reaction support as claimed in [any of the preceding] claim[s] 1 for optical analysis of the hybridization of synthesized polymeric probes with complementary fragments.

9. (Amended) The use of a microfluidic reaction support as claimed in [any of the preceding] claim[s] 1 for efficient highly parallel combined wet-chemical and light-controlled synthesis of oligomeric or polymeric probes such as DNA, RNA, PNA, LNA, proteins and also for subsequent optical analysis of the hybridization with complementary fragments.

10. (Amended) The use of a microfluidic reaction support as claimed in [any of the preceding] claim[s] 1 for light-controlled synthesis of oligomeric or polymeric

probes such as DNA, RNA, PNA, LNA and also for subsequent optical analysis of the hybridization with complementary fragments.

13. (Amended) The use of a microfluidic reaction support as claimed in [any of the preceding] claim[s] 1, in which the walls between the feed channels (2; 102; 202) and the discharge channels (3; 103; 203 are made lightproof.

14. (Amended) The use of a microfluidic reaction support as claimed in [any of] claim[s] 2 [to 11], in which the connecting channels (104; 204) consist of a multiplicity of glass fiber bundles fused together from which the glass fiber cores have been etched out, thus resulting in microchannels.

16. (Amended) The use of a microfluidic reaction support as claimed in [any of] claim[s] 2 [to 15], in which the levels consist of a silicon layer into which a multiplicity of small channels has been etched.

17. (Amended) The use of a microfluidic reaction support as claimed in [any of] claim[s] 2 [to 16], in which a plurality of levels with flow channels are arranged on top of one another such that the reaction areas in the projection perpendicular to the flow levels are not superimposed and can be photoactivated individually by light and light can be detected, likewise location-specifically, for each of the reaction areas.

18. (Amended) The use of microfluidic reaction support as claimed in [any of] claim[s] 1 [to 16], in which a programmable light source matrix is integrated into the reaction support.

19. (Amended) The use of a microfluidic reaction support as claimed in [any of] claim[s] 1 [to 18], in which a detection unit in the form of a CCD matrix is integrated into the reaction support.

20. (Amended) The use of a microfluidic reaction support as claimed in [any of the preceding] claim[s] 1, in which a multiplicity of in each case different receptors is bound to specific areas to the support.

22. The use of a microfluidic reaction support as claimed in claim 20 [or 21], in which the receptors have been synthesized on the support from individual synthesis building blocks.

23. (Amended) The use of a microfluidic reaction support as claimed in [any of] claim[s] 20 [to 22], in which a building block is introduced between receptor and support, which allows the receptor to be removed by cleavage.

25. (Amended) The use of a microfluidic reaction support as claimed in [any of] claim[s] 5 [to 24], in which luminescence and fluorescence are measured through the transparent cover layer in a backlight process.

26. (Amended) The use of a microfluidic reaction support as claimed in [any of] claim[s] 5 [to 25] with covering on both sides by transparent cover layers, in which each reaction area is exposed to light of a defined wavelength via a programmable light source matrix and is biochemically functionalized via the light and the supply of fluid and all processes in the reaction support are optically monitored simultaneously via the second transparent cover layer.

27. (Amended) The use of a microfluidic reaction support as claimed in [any of] claim[s] 5 [to 26] with covering on both sides by transparent cover layers, in which luminescence and fluorescence and also absorption are measured through the two transparent cover layers in a transmitted-light process.

28. (Amended) The use of a microfluidic reaction support as claimed in [any of the preceding] claim[s] 1 for individual wetting and biochemical functionalization of each reaction area in the reaction support.

31. (Amended) The microfluidic reaction as claimed in claim 29 [or 30], in which the fluid feed channels (202) cross the fluid discharge channels (203) at an angle in a projection perpendicular to the first and second level.

32. (Amended) The microfluidic reaction support as claimed in claim 29 [or 30], in which the fluid discharge channels (102) run parallel to the fluid feed channels (103).

PCT/EP00/07445
FeBiT Ferrarius Biotechnology GmbH
21119P WO/Tlct

Claims

1. The use of a microfluidic reaction support containing a flow channel structure for directing fluids for the synthesis of oligomers or polymers, in which the flow channel structure contains reaction areas (4; 104), fluid feed channels (2; 102) for feeding fluid to the reaction areas (4; 104) and fluid discharge channels (3; 103) for discharging fluid from the reaction areas (4; 104) and in which the reaction areas are formed by connecting channels which connect the fluid feed channels (2; 102) with fluid discharge channels running essentially parallel thereto and which are arranged at an angle to said fluid feed channels and fluid discharge channels such that fluid can be discharged from each reaction area (4; 104), with circumvention of the particular other reaction areas (4; 104).

2. The use of a microfluidic reaction support as claimed in claim 1, in which the fluid feed channels (102) run essentially parallel to one another and are in a first level of the microfluidic reaction support, in which the fluid discharge channels (103) are located in a second level of the microfluidic reaction support and in which the connecting channels with the reaction areas (104) are located perpendicular or nearly perpendicular to said levels.

3. The use of a microfluidic reaction support containing a flow channel structure for directing

fluids for the synthesis of oligomers or polymers, in which the flow channel structure contains reaction areas (204), fluid feed channels (202) running essentially parallel to one another in a first level of the reaction support for feeding fluid to the reaction areas (204) and fluid discharge channels (203) running essentially parallel to one another in a second level of the reaction support for discharging fluid from the reaction areas (204), in which the reaction areas (204) are formed by connecting channels which connect the fluid feed channels (202) with fluid discharge channels (203) and run perpendicular or nearly perpendicular to the two levels so that from each reaction area (204) fluid can be discharged with circumvention of the particular other reaction areas (204), and in which the fluid feed channels (202) cross the fluid discharge channels (203) at an angle in a projection perpendicular to the first and second level.

4. The use of a microfluidic reaction support as claimed in any of the preceding claims, in which each flow channel (2, 3; 102, 103; 202, 203) can individually be charged with fluid and discharged via a valve system.

5. The use of a microfluidic reaction support as claimed in any of the preceding claims, in which the flow channel structure is provided on one side or on both sides with a transparent cover layer.

6. The use of a microfluidic reaction support as claimed in any of the preceding claims for wet-chemical synthesis of oligomeric or polymeric probes such as DNA, RNA, PNA, LNA, and others.

7. The use of a microfluidic reaction support as

claimed in any of the preceding claims for integrated synthesis and analysis of polymers.

8. The use of a microfluidic reaction support as claimed in any of the preceding claims for optical analysis of the hybridization of synthesized polymeric probes with complementary fragments.

9. The use of a microfluidic reaction support as claimed in any of the preceding claims for efficient highly parallel combined wet-chemical and light-controlled synthesis of oligomeric or polymeric probes such as DNA, RNA, PNA, LNA, proteins and also for subsequent optical analysis of the hybridization with complementary fragments.

10. The use of a microfluidic reaction support as claimed in any of the preceding claims for light-controlled synthesis of oligomeric or polymeric probes such as DNA, RNA, PNA, LNA and also for subsequent optical analysis of the hybridization with complementary fragments.

11. The use of a microfluidic reaction support as claimed in claim 5, in which the flow channel structure is provided on both sides with a transparent cover layer, in which the transparent cover layers consist of glass or plastic and a structure of microlenses is integrated into said cover layers such that the incident light is focused on the reaction areas and the reflected light of a detection reaction is concentrated accordingly.

12. The use of a microfluidic reaction support as claimed in claim 5, in which the transparent cover layers consist of a multiplicity of parallel fused glass fibers which form a transparent honeycomb

structure such that the incident light and the reflected light are parallelized and the light is prevented from spreading sideways, due to reflection, in the cover layer.

13. The use of a microfluidic reaction support as claimed in any of the preceding claims, in which the walls between the feed channels (2; 102; 202) and the discharge channels (3; 103; 203) are made lightproof.

14. The use of a microfluidic reaction support as claimed in any of claims 2 to 11, in which the connecting channels (104; 204) consist of a multiplicity of glass fiber bundles fused together from which the glass fiber cores have been etched out, thus resulting in microchannels.

15. The use of a microfluidic reaction support as claimed in claim 14, in which the glass fiber bundles with cores etched out are arranged in the area of the reaction area.

16. The use of a microfluidic reaction support as claimed in any of claims 2 to 15, in which the levels consist of a silicon layer into which a multiplicity of small channels has been etched.

17. The use of a microfluidic reaction support as claimed in any of claims 2 to 16, in which a plurality of levels with flow channels are arranged on top of one another such that the reaction areas in the projection perpendicular to the flow levels are not superimposed and can be photoactivated individually by light and light can be detected, likewise location-specifically, for each of the reaction areas.

18. The use of a microfluidic reaction support as claimed in any of claims 1 to 16, in which a programmable light source matrix is integrated into the reaction support.

19. The use of a microfluidic reaction support as claimed in any of claims 1 to 18, in which a detection unit in the form of a CCD matrix is integrated into the reaction support.

20. The use of a microfluidic reaction support as claimed in any of the preceding claims, in which a multiplicity of in each case different receptors is bound to specific areas to the support.

21. The use of a microfluidic reaction support as claimed in claim 20, in which the receptors are selected from the group consisting of nucleic acids such as DNA, RNA, nucleic acid analogs such as peptide nucleic acids (PNA), peptides and saccharides.

22. The use of a microfluidic reaction support as claimed in claim 20 or 21, in which the receptors have been synthesized on the support from individual synthesis building blocks.

23. The use of a microfluidic reaction support as claimed in any of claims 20 to 22, in which a building block is introduced between receptor and support, which allows the receptor to be removed by cleavage.

24. The use of a microfluidic reaction support as claimed in claim 23, in which, after the receptor has been removed by cleavage, a functional group is retained on the support which is suitable for synthesis of a new receptor.

25. The use of a microfluidic reaction support as claimed in any of claims 5 to 24, in which luminescence and fluorescence are measured through the transparent cover layer in a backlight process.

26. The use of a microfluidic reaction support as claimed in any of claims 5 to 25 with covering on both sides by transparent cover layers, in which each reaction area is exposed to light of a defined wavelength via a programmable light source matrix and is biochemically functionalized via the light and the supply of fluid and all processes in the reaction support are optically monitored simultaneously via the second transparent cover layer.

27. The use of a microfluidic reaction support as claimed in any of claims 5 to 26 with covering on both sides by transparent cover layers, in which luminescence and fluorescence and also absorption are measured through the two transparent cover layers in a transmitted-light process.

28. The use of a microfluidic reaction support as claimed in any of the preceding claims for individual wetting and biochemical functionalization of each reaction area in the reaction support.

29. A microfluidic reaction support for the synthesis of oligomers or polymers, having a flow channel structure for directing fluids, in which the flow channel structure contains reaction areas (104; 204), fluid feed channels (102; 202) running essentially parallel to one another in a first level of the reaction support for feeding fluid to

the reaction areas (104; 204) and fluid discharge channels (103; 203) running essentially parallel to one another in a second level of the reaction support for discharging fluid from the reaction areas (104; 204) and in which the reaction areas are formed by connecting channels which connect the fluid feed channels (102; 202) with fluid discharge channels (103; 203) and which run perpendicular or nearly perpendicular to the two levels so that from each reaction area (104; 204) fluid can be discharged with circumvention of the particular other reaction areas (104; 204).

30. The microfluidic reaction support as claimed in claim 29, in which each flow channel (102; 202, 103; 203) can be charged with and emptied of fluid individually via a valve system.

31. The microfluidic reaction support as claimed in claim 29 or 30, in which the fluid feed channels (202) cross the fluid discharge channels (203) at an angle in a projection perpendicular to the first and second level.

32. The microfluidic reaction support as claimed in claim 29 or 30, in which the fluid discharge channels (102) run parallel to the fluid feed channels (103).

19/1PRTS
10/030182
JC03 Rec'd PCT/PTO 01 FEB 2002
WO 01/08799 PCT/EP00/07445

MICROFLUIDIC REACTION SUPPORT HAVING THREE FLOW LEVELS AND A TRANSPARENT COVER LAYER

The present invention relates to a microfluidic reaction support which, depending on the embodiment, makes possible a purely fluidic or else light-controlled synthesis and analysis of oligomers or polymers. In addition, any other application as miniaturized chemical or biochemical synthesis and analysis platform, for example for application in combinatorial chemistry, is in principle conceivable.

In general, the development of microfluidic systems is still in its infancy. However, even now they represent an important field, for example, in the area of micropumps or microvalves. The focus of present studies in this field is on the preparation of miniaturized structures, preferably using semiconductor technology methods.

Micrometering systems link microminiaturized pumps and valves to sensors for drive and regulator circuits. Such systems are currently developed and tested for specific applications, for example for dosing of medicaments or metering of very small amounts of liquids in a free jet according to the inkjet printer principle. These are used, for example, for preparing "polymeric probe arrays" by spraying various biochemical substances on defined positions of a support body.

The mixing of media in microfluidic systems, for example in chemical microreactors or in bioreactors but also in chemical analysis systems, has not been extensively studied to date. If very rapid mixing is required, however, the use of specifically constructed vortex zones or the use of a likewise miniaturized mixer can achieve very high mixing rates. The

development of micromixers is not market-ready yet and is for the most part still in the experimental stage. The interaction of fluid and wall, which is important for the microfluidic reaction support of the invention, has not to date been studied in detail.

Complete microfluid analysis systems have been realized previously only in some cases, for example in systems for analyzing the heavy metal content of ground water. Test samples and functional samples of such microfluid analysis systems are prepared by using preferably various established silicon technologies such as, for example, isotropic and anisotropic etching.

A great disadvantage of silicon technology is the relatively high cost of material. For this reason, various inexpensive technologies are currently developed, which allow preparation of microstructures as "throw-away articles". Three of these methods are micro injection molding, miniaturized hot molding and "LIGA" (light-induced galvanomolding) technology. These methods allow in the experimental stage the preparation of microstructures with dimensions of less than 1 μm.

These developments are presently applied, for example, in DNA analysis. The current subject of research here is a very rapid and therefore highly parallel detection. The combination of hybridization as detection principle and optical signal detection is in the most advanced stage. In the USA, enormous resources are being used to advance the development of these miniaturized detection chips. The analytical performance here is in the range from 10^4 to a maximum of 10^5 bases per hour.

The aim is therefore to develop a technology with the aid of which it is possible to analyze about and greater than 10^5 bases per hour and to process the obtained data such that meaningful interaction between

user and the device to be used is possible. The core of such a device is the subject of the present invention and is described as microfluidic reaction support below. This reaction support of the invention is intended to form, for example, the central component of systems for automatic fragment synthesis and fragment analysis of oligomers or polymers. A system of this kind is described in the patent application 19924327.1.

The reaction support of the invention includes a structure of microchannels of different size, geometry and function. Part of the microchannels serves to supply and discharge fluid. All other channels serve as reaction areas, and it is also possible, depending on the application, to integrate optionally fluid reservoirs, etc. into the microstructure. The flow through the reaction support is either two-dimensional or three-dimensional. The two-dimensional design variant comprises at least in each case one feed and one discharge channel in a single flow level. These two channels are connected by a plurality of channels which run approximately perpendicular thereto, and these perpendicular connecting channels serve as preferred reaction areas. The thus resulting reaction channels can likewise be divided again into smaller channels, each reaction channel comprising one or more reaction areas. These reaction areas may be arranged, for example, along the channel.

The more complex three-dimensional design variant comprises three flow levels. The feed channels are arranged in each case parallel to one another in a first flow level and the discharge channels are arranged in each case parallel to one another in the third flow level, and feed and discharge channels are arranged in a perpendicular projection either parallel to one another or at an angle to one another, said angle being chosen preferably as approximately 90°. Moreover, perpendicular channels which form a third

flow level and connect the feed channels of the first level with the discharge channels of the third level are arranged in the angled arrangement at the crossover points of the channels in their perpendicular projection or in the parallel arrangement along the channels. Said connecting channels are substantially narrower than the feed and discharge channels. This makes it possible for fluid to flow over the reaction areas in the feed and discharge channels without entering the reaction channels. Several reaction channels together form a reaction area.

Thus, the technical preconditions for a very rapid, efficient and thus inexpensive provision of a multiplicity of reaction areas have been created, for example for the integrated synthesis of a multiplicity of polymeric probes and the analysis of a multiplicity of polymer fragments by means of said probes.

In all design variants, the fluids are discharged from the reaction areas, without said fluids coming into contact with another reaction area of the entire reaction support. This is especially relevant in reactions whose waste products could damage or destroy other reaction areas.

All three variants of the microfluidic reaction support of the invention have a cover layer on both the top and the bottom. In the case of the two-dimensional structure and also in the case of the parallel feed and discharge channels of the three-dimensional structure, at least one of the cover layers has a transparent structure in order to make possible a light-controlled photoactivation in the individual reaction areas by individual illumination, for example by means of a programmable light source matrix as described in the patent application 199 07 080.6. All three variants are constructed preferably with two cover layers, in order to make possible a permanent optical process control in

the reaction support and measurement of detection reactions in transmitted light.

Various protective groups which are partly used also in the synthesis of microarrays are known and available for light-dependent photoactivation. Examples of protective groups included here are MeNPOC, NPPOC and its derivatives and also some older protective groups which have been described by Pillai (Synthesis, 1980); Hadrisan and Pillai (Proc. Indian nat. Sci. Acad. 53, 1987) or Birr et al (Liebigs Ann. chem. 763, 1972).

Moreover, methods in which photoactivation leads indirectly via light-dependent activation of an acid (photo acid) to a subsequent location-specific removal of an acid-labile protective group such as, for example, DMT are also known (see Gao et al in WO 9941007). A similar mechanism can be utilized if suitable photoresists are applied to the reaction support (see McGall in PNAS 93, pp. 13555-13560, 1996).

In addition to these chemical methods it is also conceivable to control synthesis by photoactivation and photodeactivation of enzymes.

The more complex three-dimensional structure containing the feed and discharge channels rotated at an angle makes it possible to individually rinse each individual reaction area of the vertically arranged microchannels. This is carried out by rinsing in each case one feed channel with fluid and discharging fluid through one discharge channel. The fluid flows through the feed channel into the perpendicular microreaction channels and out of the reaction support again through the discharge channel. In the same way it is possible to rinse a plurality of reaction areas at the same time and even with different fluids. Thus the microfluidic reaction support of the invention, which has a "cruciform structure" due to the angled arrangement,

opens up a multiplicity of applications of combinatorial chemistry or DNA analysis.

Another application is suffusing initially all feed and discharge channels alternately with starting materials, with the fluid supply and fluid discharge functions of the feed and discharge channels alternating from cycle to cycle. If, for example, each channel is rinsed with a different building block of a polymeric probe to be synthesized, then it is possible to generate over a few cycles a large variety of oligomeric or polymeric probes in the individual reaction areas of a reaction support, due to the use of the cruciform structure. Additionally, the synthesis of individual probes of any specificity in a single reaction area is possible without problems by individually driving a reaction area as described above. Thus the inventive microfluidic reaction support with cruciform structure provides the possibility of efficient wet-chemical "probe array" synthesis of oligomeric or polymeric probes. This procedure is denoted "fluidic multiplexing" hereinbelow. This also makes possible in-situ synthesis by means of process monitoring and also integrated synthesis and analysis.

The purely fluidic reaction control requires no transparent cover layers which are, however, likewise sensible for optical process control and for recording detection reactions. In this case, detection may be carried out likewise either in transmitted light or else in back light from one side. If the three-dimensional cruciform structure with its feed and discharge channels arranged by rotation at an angle is combined with the light-controlled photoactivation of the reaction areas of microchannels, the efficiency of synthesizing oligomeric or polymeric probes can be increased still further. It is possible to integrate both the light source matrix as light source and the required detector into the microfluidic reaction

support. The same can be said for integrating a CCD matrix as second opposite cover layer. It is also possible to connect a programmable light source matrix as cover layer directly. This suggests itself, in particular if the microfluidic reaction support is integrated into a device as a fixed component and is purified, for example chemically, between uses and has only to be changed for maintenance purposes. If the microfluidic reaction support is exchanged after each use, then, however, direct integration is not sensible. In this case, it is recommended rather to arrange the components in the system accordingly.

The invention likewise relates to supplying the microfluidic reaction support with the appropriate fluids. For this purpose a likewise novel integrated valve system was designed. This allows rapid provision of a multiplicity of fluids in the feed and discharge channels of the microstructure.

This fluid supply system has been designed for applying the microfluidic reaction support of the invention to the synthesis of arrays of oligomeric or polymeric probes in the reaction areas. The supply system is similar in the connections and components for the "upper" and "lower" feed and discharge channels. All channels are individually supplied from one side via a multiplex valve described below. All channels are combined at the in each case corresponding other end of the channel, and this combining is used for feed and discharge with uniform rinsing of all reaction areas. When synthesizing oligomeric or polymeric probes in the reaction areas, this refers to all cycles except for feeding the specific individual building blocks consisting of, for example, one or more nucleotides in the case of DNA synthesis. If it is intended to reach all reaction areas and not to select them specifically, then it is better to choose a flow-optimized feed such as, for example, dual ramification, rather than via the

multiplex valve, which has a higher risk of delay. However, the valve is required for feeding the specific building blocks. Said valve connects the microchannels of the reaction support on one side with an at most identical number of individual tanks and a group connection on the other side. In one valve position, in each case one tank is connected with one or more channels of the reaction support. If the fluid of a single tank is intended to enter more than one channel or channel bundle of the reaction support in a single cycle, first one channel and then further channels are provided in series. The group connection corresponds to the combination of the channels on the in each case opposite side of the reaction support. It serves to efficiently rinse valve and reaction support.

The connections of the microfluidic reaction support to its fluid supply and fluid disposal are an important element. If the reaction support is purified time and again and reused in the specific application, a complicated connection technique, for example to the multiplex valve, may be provided for. In this case, in particular in the case of a large number of channels, a design with a multiplicity of very small channels in "legs", in analogy to semiconductor processor technology, is possible. The disadvantage of this design with respect to flow is the risk of deposits in the bends and kinks of the individual microchannels. Here, subsequent rinsing may be provided for, as for avoiding delays. For the application variant in which the reaction support is exchanged after each application, rapid connections which seal without adhesive are required. In this connection, it is possible, for example, to connect flat to the front of the reaction support, with a through bend-free channel course. Thus the risk of delay is minimal. A second alternative is pressing the bottom of the reaction support onto the fluid feed. In this connection, suitable seals resistant to chemicals have to be

provided for in each case.

In one aspect of the invention, purification means in particular a complete regeneration of the reaction support. Said support can then be used again in the regenerated state for a new polymer synthesis. In the case of chemical purification, it must preferably be taken care that the linkage site required for attaching a first polymer building block is not destroyed. The predetermined breaking point necessary for chemical purification may be cleaved by chemical (e.g. wet-chemical, photo-chemical, electrochemical) or biological (e.g. enzymic) transformation. Preference is given to providing the predetermined breaking point during the first surface derivatization of the microfluidic reaction support, preferably in the linker system which connects the surface to the first polymer building block. In each case it is guaranteed that the predetermined breaking point cannot be broken by the analyte or reagents used during synthesis or during analysis.

One-stage process:
The predetermined breaking point is broken by a single transformation. Examples of this are base-labile linkers, acid-labile linkers, oxidation-labile linkers or degradation with the aid of suitable enzymes.

Apart from chemical purification, it is thus also possible to carry out an enzymic purification of the reaction support. In this case, the polymeric or oligomeric probes linked to the reaction support are cleaved or "digested" with a DNA- or RNA-degrading enzyme or a peptide-cleaving enzyme, resulting in degradation of a part or all of the probes. Afterward, the reaction support can be used again for synthesizing new probes.

Suitable enzymes are nucleases such as exonucleases or

endonucleases which attack one nucleic acid strand from the ends or within the probe strand and which leave behind nucleotides or nucleosides as cleavage products. In the case of RNA, it is possible to use RNAses (RNAse H, etc.) which, if an RNA-DNA double strand has been generated, selectively cut the RNA part resulting in cleavage of the entire probe in the case of RNA probes and of the RNA section in the case of RNA part sections as predetermined breaking point. Likewise, a reaction support can be regenerated with DNA probes by using DNAses (DNAse I, DNAse II, etc.), and, as a result, both single-stranded and double-stranded DNA can be degraded.

Likewise, it is possible to use peptide-cleaving enzymes for degradation of peptide probes or peptide sequence sections as predetermined breaking point.

Multistage process:
The predetermined breaking point is broken in a multistage process, i.e. the predetermined breaking point is masked in some form. This requires firstly removing said masking in one or more steps before in the subsequent step the predetermined breaking point can then finally be broken.

As an example a masked photolabile linker can be used, in which an o-nitro function required for photolability is only generated by a preceding transformation. This may take place, for example, by oxidation of an amino function. This, not necessarily specific, oxidation step may be carried out enzymatically or wet-chemically. Once the o-nitro function has been generated, it is then possible for the predetermined breaking point to be cleaved by irradiation with light.

Another possible solution is to generate in a first step a double-stranded DNA sequence by adding an analyte complementary to the linker, which is then in

the next step recognized by a specific enzyme (restriction enzyme) and is removed by specific cleavage.

When using an RNA part section as probe "base", it is possible to chemically regenerate the reaction support likewise in several stages. In this connection, the synthesis is initially carried out using 2'-OH-protected phosphitamide building blocks. After hybridization and analysis, the protective group is regenerated by cleaving off the RNA part section, resulting in a free 2'-OH group. This may be followed in a subsequent chemical reaction step by cleavage of the ribose sugar with the aid of periodate or other oxidants and removal of the probe from the reaction support by β elimination.

The above-described purification (receptor removal) processes have independent significance within the scope of the invention, irrespective of a specific embodiment of the support. The applicant reserves putting forward, where appropriate, independent patent claims regarding the described technology of purification or receptor removal.

It should be pointed out that it is also possible to remove the receptor or molecule by cleavage in the sense explained above, in order to collect molecules removed by cleavage and to use said molecules for further chemical processes, for example for a synthesis step. In this sense, the purification processes may be seen as steps for obtaining molecules synthesized on a support.

The microfluidic reaction support of the invention is constructed in several layers, as is common also in semiconductor microtechnology. In this case it is possible to distinguish between dividing the microstructure into functional layers and into layers

due to the construction.

While a two-dimensional structure has at least three functional layers, a three-dimensional structure consists of at least five functional layers. These functional layers are described in more detail below. During production is it often possible to integrate a plurality of these functional layers by means of suitable production methods into a layer due to the construction.

The functional layers of the two-dimensional structure contain a central structural layer into which the microflow structure of channels, reaction areas and reservoirs is introduced. It is connected with an upper and a lower cover layer and may be made of glass, plastic or silicon. Depending on the design, the material used may be transparent or else lightproof. An example of lightproof material, which is recommended, is Futoran glass from Schott, silicon or Teflon.

The three-dimensional structures consist of five functional layers, a first, "upper" cover layer, a structure of microchannels, located underneath said cover layer, for feeding and discharging fluid in a manner analogous to the two-dimensional structure, a central level of perpendicular, smaller (preferably by at least a factor of 10) microchannels which serve as reaction areas. At the "bottom" a level for fluid supply and a cover layer follow, both of which are designed similarly to the "top". Overall, the reaction support is a construction mirrored at a central plane. The preparation need not necessarily follow the functional layers. Thus it is possible to integrate the feed and discharge structure both into the central layer and into the cover layer. It is possible to use for the central layer with the perpendicular microchannels as reaction areas, for example, suitable silicon wafers from the semiconductor technology, which

has etched "pores", from Siemens or fused glass fibers (glass fiber wafers) from Schott, having etched-out cores and a size ratio between wall thickness and channel diameter of preferably 1 to 5. In order to improve precise rinsing of only the "driven" reaction channels, it is possible to supplement the central functional level with an upper and a lower intermediate layer. Said layer prevents or makes more difficult unwanted streaming-in of fluids (hydrophilic or hydrophobic barriers).

The preparation methods required can be distinguished according to the material used. In the case of silicon wafers, glass wafers and glass fiber wafers (with and without core), the connection techniques used are bonding methods. The parts such as, for example, the various wafers are prepared by etching techniques and also sawing and polishing. When plastics such as Teflon which is lightproof and COC or polystyrene which is transparent are used, methods such as injection molding, hot molding or LIGA are used. The components are connected, for example, by means of adhesive bonding or ultrasound welding or by mechanical pressure sealing by means of a holder or a frame.

The upper cover layer seals on the outside the microflow structure lying underneath. This produces the microchannels. The layer is transparent for introducing light into said channels. In order to optimize the optics, it is also possible to use microlenses made of glass from Mikroglas or plastic (IMM Mainz). Likewise possible is the use of a honeycomb structure made of fused glass fibers which was developed, for example, by Schott or ITT and is used, for example, in night vision equipment. To this end, long glass fiber bundles are heated such that they fuze and [illegible]. These may then be bonded to glass or silicon or bonded or welded to plastics.

The proper use of the microfluidic reaction support of the invention is as follows: firstly a group of reaction areas is addressed via the microchannels of a two- or three-dimensional microstructure. After the reaction has taken place there, the reaction products forming in the individual reaction areas are discharged through microchannels, without the reaction product flowing through another reaction area. In this connection, driving of the reaction areas in the described three-dimensional cruciform structure may be utilized for purely fluidic synthesis of oligomers or polymers from monomers, oligomers or polymers or else for accelerating the light-controlled synthesis or a combined wet-chemical and light-controlled synthesis of oligomers or polymers by the described intelligent multiplexing of the starting materials.

In the meantime, all reaction areas and microchannels are optically controlled through transparent cover layers, this being a platform for an in-situ synthesis, a permanent process control and regulation of the processes in the microstructure. This creates the basis for a comprehensive quality assurance. Light signals of detection reactions, which are produced in the reaction areas by chemical (e.g. luminescence), biochemical (e.g. bioluminescence) or light-induced (e.g. fluorescence) reactions, can be recorded in an integrated apparatus for synthesis and analysis, which encloses the fluidic microprocessor and is described in the patent application 19924327.1. Furthermore, absorption can be measured in the reaction support by recording light signals which pass through the microchannels and reaction areas in a transmitted-light process or are reflected in a backlight process. This may be utilized, for example, for an extended qualitative quality assurance.

This microfluidic reaction support of the invention has many different advantages: first, the reaction products

are discharged from each reaction area without another reaction area coming into contact with the reaction products. This makes it possible to carry out reactions for synthesis and analysis in those reaction areas which generate reaction products (final products or intermediates) which would be harmful to other reaction areas.

Compared with planar surfaces, the three-dimensional microchannels have a larger surface area which can be utilized as a solid phase.

The use of microstructures reduces the amount of fluid required for the reactions and at the same time increases the reaction rate. This is the case both for covalent bonds and, for example, for the hybridization times for applications in DNA, RNA, PNA, LNA analysis or for protein applications.

Transparent cover layers make possible photoreactions, for example for the light-controlled synthesis of DNA, RNA, PNA, LNA or proteins, etc.

Moreover, the transparent cover layers make possible a permanent process control for regulating the reactions and also the fluidics in the reaction support. This leads to a distinct reduction in the mistakes both in production and in detection, thus increasing the number of analyzable measurements per use of material and time.

A suitable layout of the geometry of the individual reaction areas and the microchannels between the reaction areas makes it possible to specifically influence the beam paths, taking into account the refraction indices present in the reaction support.

The fluidic microprocessors of the invention may be designed as simple components for single use. In

principle inexpensive plastic structures are to be preferred here but possible designs are also glass and silicon [lacuna] or else combinations of materials. Rapid and inexpensive production makes possible a large variety of individual applications in which probe arrays can be synthesized and analyzed specifically, for example by taking into account sequence and gene databases on the Internet.

The reactions always take place on the walls of the microreaction channels. Consequently, the reaction areas always have a three-dimensional structure and have a considerably larger surface area than the planar base area. This three-dimensional geometry thus greatly enlarges the utilizable reaction surface area. Said size of the surface area is very important for the use as solid phase. Said size may be important, for example, for accumulation of oligonucleotides during synthesis in the reaction support as well as for accumulation of sample fragments flowing past during an analysis in the reaction support.

The three-dimensional cruciform structure makes applications, for example, in oligonucleotide analysis or in combinatorial chemistry, etc. possible. Using the two intersecting structures makes it possible to generate quickly a multiplicity of different combinations of oligomers or polymers in the individual reaction areas of the reaction support. This makes possible a very efficient wet-chemical synthesis of an oligomeric or polymeric probe array in a reaction support. This may be controlled by a computer, making it possible to generate any nucleotide combinations in each reaction area. The analysis may likewise be carried out directly in the reaction support, making permanent process control possible.

An appropriate multiplexing of the fluids can reduce the number of preparation cycles of "probe arrays". The

location-specific generation of a multiplicity of different oligomeric or polymeric probes of, for example, 20 bases in length on a planar surface by means of local photoactivation requires on each level four synthesis cycles, due to the four different bases. Thus a total number of $4 \times 20 = 80$ cycles is required. There is no systematic possibility of reducing the number of synthesis cycles. On the other hand, synthesis in the microfluidic reaction support offers the possibility of distributing the starting materials, i.e. monomers or oligomers, simultaneously on microfluidic subareas. As a result, it is possible to reduce the synthesis cycles, for example when using tetramers, to a minimum of 5 cycles. The exact number of cycles required for a specific probe array is specific for each probe pattern and can be stated only as statistical average, if the number of reaction areas in the reaction support, the number of parallel fluidic subspaces and the length of the oligomers to be synthesized are given.

The following methods become applicable during the reaction support of the invention: apart from the synthesis of oligomers and polymers up to whole genes and genomes, there is the possibility of "de novo" sequencing of unknown polymers such as DNA, RNA, PNA, LNA, proteins and others via sequence comparison with processed sample material. In addition, it is possible to "re"-sequence polymers, i.e. to compare known with unknown sequences, the known sequences being specifically selected. Likewise, it is possible to prepare substance libraries for screening methods and analytical methods, in particular for nucleic acid analysis via hybridization.

All processes from synthesis to analysis of simple or complex molecules can be integrated in the microfluidic reaction support of the invention and can be carried out very efficiently. This makes possible, for example,

the flexible and cost-saving analysis of a large number of polymers by providing a multiplicity of individual and specific polymeric probes in a miniaturized format and subsequent comparison of the probes with analytes of the sample material. This makes it possible to generate in screening methods and analytical methods a large number of measurement data and thus to deal with the wealth of information of biological systems efficiently and in its entirety in a very short time.

Fields of application are also methods and devices for the continuous, discrete fragment analysis, which are accelerated by the present invention and therefore made efficiently usable, and, in principle, all applications of oligomer/polymer analysis, as in liquid chromatography/high pressure liquid chromatography, gas chromatography, thin layer chromatography, gel electrophoresis, capillary electrophoresis, mass spectrometry, etc. and also all "probe array" applications. Furthermore supported is thus substance development and testing of appropriate substances, inter alia in pharmaceutical research. Further important fields of application are molecular diagnostics, DNA and/or RNA analysis, screening for molecular interactions, for example in immunology, molecular biology, histology and combinatorial chemistry.

There is a multiplicity of design variants in the design as well as in the production of the reaction supports, which are depicted in the following drawings:

Fig. 1a shows a two-dimensional structure of the microfluidic reaction support in plan view. Fig. 1b and 1c show the corresponding sectional illustrations: the microchannel structure 1 is located in the central flow level 30 of the reaction support. Said central flow level is sealed by the lower cover layer 10 and the

upper cover layer 20. The flow structure consists of feed channels 2 and discharge channels 3 and also of the reaction channels 4 located in between and having in each case at least one reaction area.

Fig. 2 shows a three-dimensional structure of the microfluidic reaction support in plan view. Fig. 2b, 2c and 2d show the corresponding sectional illustrations: the microchannel structure 100 consists of the lower fluid feed structure 32 with microchannels 102 and the upper discharge channel structure 31 with the microchannels 103. In the central layer 40 in between are the connecting or reaction channels in the reaction areas 104, which are arranged nearly perpendicular to the feed and discharge. The cover layers 20 and 30 are optionally transparent or lightproof.

Fig. 3a, 3b and 3c show again the illustrations of Fig. 2a, 2b and 2c. In this case, the sectional illustrations illustrate the course of the flow through the feed channels 102, the reaction channels 101 in the reaction areas 104 and through the fluid discharge 103.

Fig. 4a shows a three-dimensional cruciform structure of the microfluidic reaction support in plan view. Fig. 4b, 4c, 4d and 4e show the corresponding sectional illustrations: the microchannel structure 200 is located in the lower fluid feed and fluid discharge structure 32 with microchannels 202 and the upper fluid feed and fluid discharge structure 31 with the microchannels 203, in each case rotated by 90° toward one another. In the central layer 40 in between are located the connecting or reaction channels in the reaction areas 204, which are

arranged perpendicular to the feed and discharge. The cover layers 20 and 30 are optionally transparent or lightproof.

Fig. 5a, 5b and 5c show once more the illustrations of Fig. 4a, 4b and 4c. In this connection, the sectional illustrations of the microstructure 200 illustrate the course of flow through the feed and discharge channels 202 and 203 and also through the reaction channels 201 in the reaction areas 204.

Fig. 6 shows the illustration of a single two-dimensional flow structure in analogy to Fig. 1 with altered cross sections of the feed channels 2 and the discharge channels 3 to specifically influence the flow. The cross section of the reaction channels 4 having in each case at least one reaction area is unaltered but may also be modified.

Fig. 7a shows in analogy to Fig. 6 a single two-dimensional flow structure in which the cross sections of the feed channels 2 and the discharge channels 3 have been altered at the level of the channels to specifically influence the flow. Here, the cross section of the reaction channels 4 having in each case at least one reaction area has likewise been altered and their size is not uniform. The structure is closed by the cover layers 10 and 20 which are arranged at an angle.

Fig. 8 shows the illustration of a three-dimensional flow structure in analogy to Fig. 2 and 3 with altered cross sections of the feed channels 102 and the discharge channels 103 to specifically influence the flow. The size of the reaction channels in the reaction areas 104 is unchanged

here.

Fig. 9 shows an illustration analogous to Fig. 8, with the size of the reaction areas 104 differing from the size of the feed channels 102 and discharge channels 103.

Fig. 10a, 10b and 10c show an illustration analogous to Fig. 3a, 3b and 3c, with the feed channels 102 and the discharge channels 103 altering their height and thus influencing the flow. Due to the thickness of the central structural layer 40, the reaction areas 104 and the reaction channels 101 have a uniform length.

Fig. 11a, 11b, 11c, 11d and 11e show a three-dimensional cruciform structure of the flow in an illustration analogous to Fig. 4a, 4b, 4c, 4d and 4e and 5a, 5b and 5c, with altered cross sections of the feed channels 202 and discharge channels 203 to specifically influence the flow. The size of the reaction channels in the reaction areas 204 is unchanged here.

Fig. 12a shows the illustration of Fig. 5c of the cruciform structure with two detail variants 12b and 12c. The detail 12b illustrates the structure composed of the cover layers 10 and 20 and a central layer 40 having the reaction areas in the reaction channels 201 and the feed channels 202 and the discharge channels 203. In detail 12c the reaction channels 201 of the variant 12b are replaced in each case by a three-layer microstructure. Said microstructure comprises two layers 301 and 303 for smoothing and stabilizing the feed flow and discharge flow 202 and 203 and also an actual reaction layer 302 made of further microchannels and, for example, a glass fleece.

Fig. 13 shows a connection variant of the microcruciform structure 200 according to Fig. 4a, 4b, 4c, 4d, 4e and 5a, 5b and 5c with two microflow channel variants 401 and 402. Both variants connect a channel for the fluid supply 400 in each case with all parallel channels 202 and 203 of the two levels. Thus it is possible to rinse all reaction areas 204 with fluid on various feed and discharge variants at the same time.

Fig. 14 shows an illustration analogous to Fig. 13 with two valves 500 integrated into the fluid supply. Said valves supply the microchannel structure 200 via the channels in the first level 202 and the second level 203. This makes it possible to rinse the reaction channels in the reaction areas 204 with fluid. It is possible to rinse one, more or all reaction areas 204 with fluid at the same time. The valve position and the direction of flow through the reaction channels make it possible to rapidly realize any fluid supply cycles. All that is required here is to change the position of the valves 500 and to apply superatmospheric or subatmospheric pressure. The uniform feeds 400, here with the channel variant 402, may also be integrated into the fluid cycles.

Fig. 15a shows a design variant of the valve 500 of Fig. 14 with further sectional illustrations 15b and 15c. The valve is designed horizontally in microtechnique. It consists essentially of a disk 509 and a plate 600. The plate is linked to the microstructure 200 via channels 601 to 604 so that optionally the fluids of the feed channels or of the microtanks behind the channels 501 to 504 can be pumped into the

channels 202 of the microstructure. The assignment can be altered in series by turning the valve disk 509. According to Fig. 14, said valve 500 can also be connected to both channel structures 202 and 203 of the cruciform structure 200. This makes it possible to wet the reaction channels individually with fluid. In analogy to the rigid junctions 401 and 402 of Fig. 13, the individual microchannels 601 to 604 are optionally connected via a central feed 510 in the valve 500, for example for uniform rinsing during purification or other uniform steps, for example during the location-resolved synthesis in the reaction support.

Fig. 16a shows another design variant of the multiplex valve 500 with the sectional illustration 16b. Here, the individual supply channels 501 to 516 are arranged in a circle around the reaction support 200. The principle corresponds to Fig. 15a, 15b, 15c. However, said design variant makes it possible to realize more or bigger connections. The disk 509 is again located on a two-layer base plate 600 and 610.

Fig. 17 shows a cross section of a fluidic reaction support which is held by a clamping device which is provided with two opposite clamping jaws 701 and 702 with an integrated flow guide 703, and said flow guide in a single flow level 202 requires no bend, etc. in the channels. The same arrangement is also possible for the channels 203. Furthermore illustrated is a narrow sealing area 705.

Fig. 18 shows another connection variant with flow guide 703 with bends 704 in at least two levels. Furthermore illustrated is a broad sealing area 705 in the support 710.

Fig. 19 shows another connection variant with flow guides 703 with bends 704 in at least two levels. Microlegs 721, analogous to a semiconductor technology processor, connect the base 720 with the reaction support 200 and the channels 202. The channels 203 can be connected analogously. A sealing is carried out through the microlegs 721 by adhesive bonding or plugging-in.

Fig. 20 shows, on the basis of the example of the microlegs 721 of Fig. 19, an after-rinse 803 for avoiding deposits in a bend of the flow and the risk of a delay connected therewith. Said microlegs 721 are anchored in the lower cover layer 10 in the reaction support. The second row of purification legs 801 makes it possible to specifically rinse the channels 802 in the corners 803 with fluid and thereby avoid or remove a deposit.

Claims

1. A microfluidic reaction support having a plurality of reaction areas, characterized in that said reaction support contains a flow channel structure for carrying fluids, and feed channels and discharge channels parallel thereto are connected to one another by connecting channels arranged at an angle to said feed and discharge channels and said connecting channels or else parts of said feed and discharge channels serve as reaction areas.

2. The microfluidic reaction support as claimed in claim 1, characterized in that the flow channel structure consists of three flow levels, the feed channels being located parallel to one another in a first flow level and the discharge channels being located parallel to one another in a third flow level and the connecting channels with the reaction areas being located perpendicular or nearly perpendicular to these two flow levels.

3. The microfluidic reaction support as claimed in claim 2, characterized in that the feed channels of the first flow level cross the discharge channels of the third flow level at an angle in a projection perpendicular to the first and third flow levels.

4. The microfluidic reaction support as claimed in any of claims 1 to 3, characterized in that each flow channel can individually be charged with fluid and discharged via a valve system.

5. The microfluidic reaction support as claimed in any of claims 1 to 4, characterized in that the fluid of each reaction area is discharged, with

said fluid making no contact with the other reaction areas.

6. The microfluidic reaction support as claimed in any of claims 1 to 5, characterized in that the flow channel structure is provided on one side with a transparent cover layer.

7. The microfluidic reaction support as claimed in any of claims 1 to 5, characterized in that the flow channel structure is provided on both sides with a transparent cover layer.

8. The microfluidic reaction support as claimed in claim 6 or 7, characterized in that the transparent cover layers consist of glass or plastic and a structure of microlenses is integrated into said cover layers such that the incident light is focused on the reaction areas and the reflected light of a detection reaction is concentrated accordingly.

9. The microfluidic reaction support as claimed in claim 6 or 7, characterized in that the transparent cover layers consist of a multiplicity of parallel fused glass fibers which form a transparent honeycomb structure such that the incident light and the reflected light are parallelized and the light is prevented from spreading sideways, due to reflection, in the cover layer.

10. The microfluidic reaction support as claimed in any of claims 1 to 9, characterized in that the walls between the feed channels and the discharge channels are made lightproof.

11. The microfluidic reaction support as claimed in any of claims 2 to 9, characterized in that the

connecting channels consist of a multiplicity of glass fiber bundles fused together from which the glass fiber cores have been etched out, thus resulting in microchannels.

12. The microfluidic reaction support as claimed in claim 11, characterized in that the glass fiber bundles with cores etched out are arranged in the area of the reaction area.

13. The microfluidic reaction support as claimed in any of claims 2 to 9, characterized in that the connecting levels consist of a silicon layer into which a multiplicity of small channels has been etched.

14. The microfluidic reaction support as claimed in any of claims 2 to 9, characterized in that a plurality of flow levels are arranged on top of one another such that the reaction areas in the projection perpendicular to the flow levels are not superimposed and can be photoactivated individually by light and light can be detected, likewise location-specifically, for each of the reaction areas.

15. The microfluidic reaction support as claimed in any of claims 1 to 14, characterized in that a programmable light source matrix for synthesis and analysis is integrated into the reaction support.

16. The microfluidic reaction support as claimed in any of claims 1 to 14, characterized in that a detection unit in the form of a CCD matrix is integrated into the reaction support.

17. The microfluidic reaction support as claimed in any of the preceding claims, characterized in that a multiplicity of in each case different receptors

is bound to specific areas to the support.

18. The microfluidic reaction support as claimed in claim 17, characterized in that the receptors are selected from the group consisting of nucleic acids such as DNA, RNA, nucleic acid analogs such as peptide nucleic acids (PNA), peptides and saccharides.

19. The microfluidic reaction support as claimed in claim 17 or 18, characterized in that the receptors have been synthesized on the support from individual synthesis building blocks.

20. The microfluidic reaction support as claimed in any of claims 17-19, characterized in that a building block is introduced between receptor and support, which allows the receptor to be removed by cleavage.

21. The microfluidic reaction support as claimed in claim 20, characterized in that, after the receptor has been removed by cleavage, a functional group is retained on the support which is suitable for synthesis of a new receptor.

22. The use of a microfluidic reaction support as claimed in any of claims 6 to 21, in which luminescence and fluorescence are measured through the transparent cover layer in a backlight process.

23. The use of a microfluidic reaction support as claimed in any of claims 7 to 22, in which each reaction area is exposed to light of a defined wavelength via a programmable light source matrix and is biochemically functionalized via said light and said supply of fluid and all processes in the reaction support are optically monitored

simultaneously via the second transparent cover layer.

24. The use of a microfluidic reaction support as claimed in any of claims 7 to 22, in which luminescence and fluorescence and also absorption are measured through the two transparent cover layers in a transmitted-light process.

25. The use of a microfluidic reaction support as claimed in any of claims 1 to 22 for wet-chemical synthesis of oligomeric or polymeric probes such as DNA, RNA, PNA, LNA, and others.

26. The use of a microfluidic reaction support as claimed in any of claims 1 to 22 for integrated synthesis and analysis of polymers.

27. The use of a microfluidic reaction support as claimed in any of claims 1 to 22 for optical analysis of the hybridization of polymeric probes with complementary fragments.

28. The use of a microfluidic reaction support as claimed in any of claims 1 to 22 for efficient highly parallel combined wet-chemical and light-controlled synthesis of oligomeric or polymeric probes such as DNA, RNA, PNA, LNA, proteins and others and also for subsequent optical analysis of the hybridization with complementary fragments.

29. The use of a microfluidic reaction support as claimed in any of claims 1 to 22 for light-controlled synthesis of oligomeric or polymeric probes such as DNA, RNA, PNA, LNA and others and also for subsequent optical analysis of the hybridization with complementary fragments.

30. The use of a microfluidic reaction support as

claimed in any of claims 1 to 22 for individual wetting and biochemical functionalization of each reaction area in the reaction support.

(12) NACH DEM VERTRAG ÜBER DIE INTERNATIONALE ZUSAMMENARBEIT AUF DEM GEBIET DES PATENTWESENS (PCT) VERÖFFENTLICHTE INTERNATIONALE ANMELDUNG

(19) Weltorganisation für geistiges Eigentum
Internationales Büro

(43) Internationales Veröffentlichungsdatum
8. Februar 2001 (08.02.2001)

PCT

(10) Internationale Veröffentlichungsnummer
WO 01/08799 A1

(51) Internationale Patentklassifikation[7]: B01L 3/00

(21) Internationales Aktenzeichen: PCT/EP00/07445

(22) Internationales Anmeldedatum: 1. August 2000 (01.08.2000)

(25) Einreichungssprache: Deutsch

(26) Veröffentlichungssprache: Deutsch

(30) Angaben zur Priorität:
199 35 433.2 1. August 1999 (01.08.1999) DE

(71) Anmelder *(für alle Bestimmungsstaaten mit Ausnahme von US)*: FEBIT FERRARIUS BIOTECHNOLOGY GMBH [DE/DE]; Käfertaler Strasse 190, 68167 Mannheim (DE).

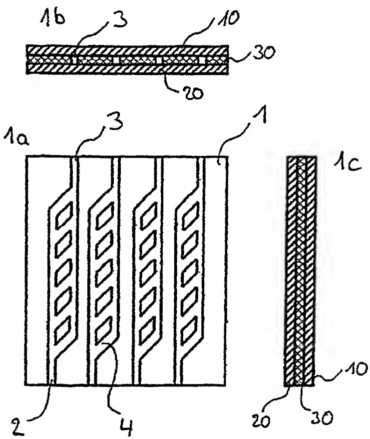
(72) Erfinder; und
(75) Erfinder/Anmelder *(nur für US)*: STÄHLER, Cord, Friedrich [DE/DE]; Siegfriedstrasse 9, 69469 Weinheim (DE). MÜLLER, Manfred [DE/DE]; Reutterstrasse 76b, 80689 München (DE). STÄHLER, Peer, Friedrich [DE/DE]; Riedfeldstrasse 3, 68169 Mannheim (DE).

(74) Anwälte: WEICKMANN, H. usw.; Kopernikusstrasse 9, 81679 München (DE).

[Fortsetzung auf der nächsten Seite]

(54) Title: MICROFLUID REACTION CARRIER HAVING THREE FLOW LEVELS AND A TRANSPARENT PROTECTIVE LAYER

(54) Bezeichnung: MIKROFLUIDISCHER REAKTIONSTRÄGER MIT DREI STRÖMUNGSEBENEN UND TRANSPARENTER DECKSCHICHT



(57) Abstract: The present invention relates to a microfluid reaction carrier intended for the purely fluid or light-controlled synthesis or analysis of oligomers or polymers. The reaction carrier comprises a structure of flow channels for the fluids, while supply channels and discharge channels parallel to the latter form an angle relative to the plane of the structure of the flow channels (reaction areas).

(57) Zusammenfassung: Gegenstand der vorliegenden Erfindung ist ein mikrofluidischer Reaktionsträger, der eine rein fluidische oder auch eine lichtgesteuerte Synthese und Analyse von Oligomeren oder Polymeren ermöglicht. Der Reaktionsträger enthält eine Strömungskanalstruktur für das Durchleiten von Fluiden, wobei Zuführungen u. zu ihnen parallele Ableitungskanäle unter einem Winkel zur Ebene der Strömungskanalstruktur (Reaktionsbereiche) liegen.

WO 01/08799 A1

Fig. 1b

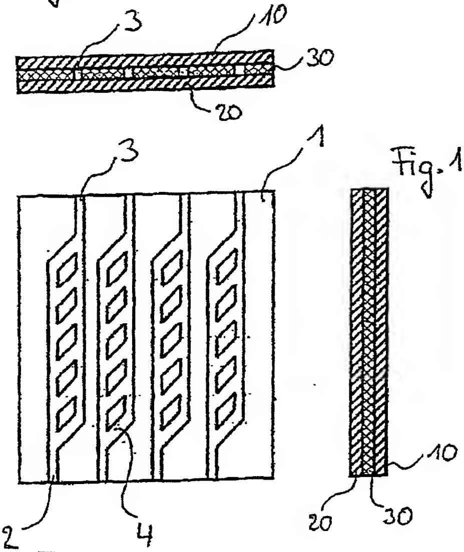


Fig. 1c

Fig. 1a

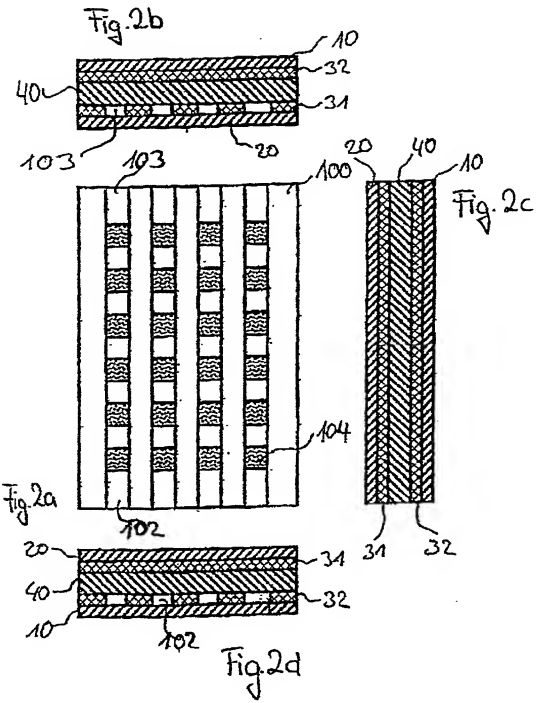


Fig. 2b

Fig. 2c

Fig. 2a

Fig. 2d

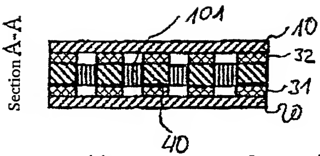
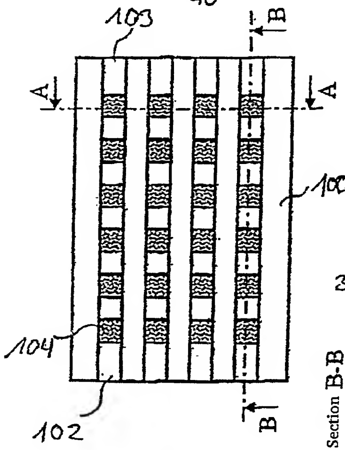


Fig. 3b



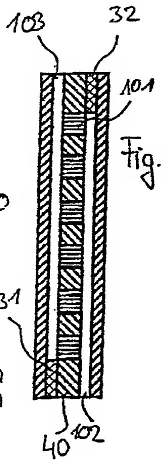


Fig. 3c

Fig. 3a

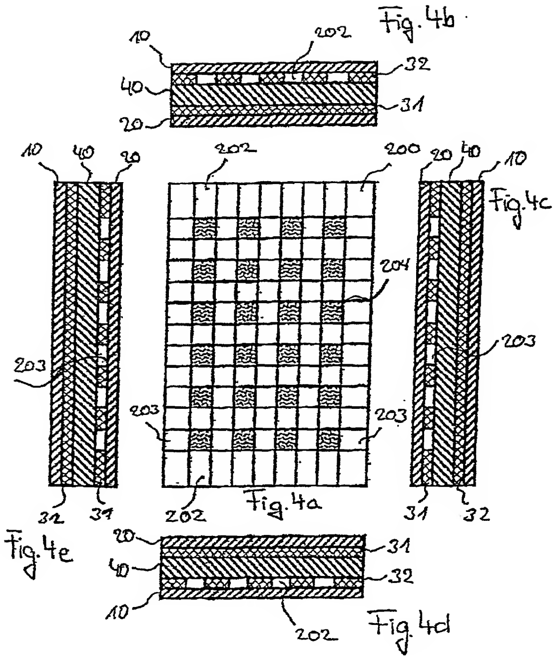


Fig. 4b
10
202
32
40
31
20
10
40
20
202
200
20
40
10
Fig. 4c
204
203
203
203
203
31
32
31
32
202
Fig. 4a
Fig. 4e
20
31
40
32
10
202
Fig. 4d

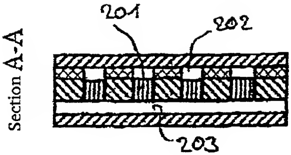


Fig. 5b

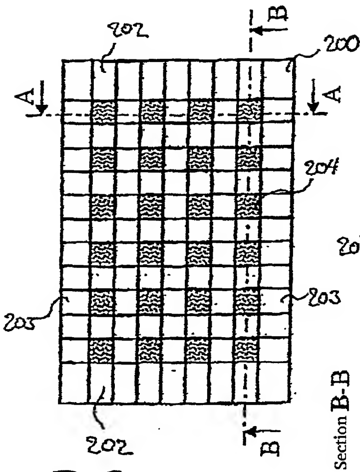
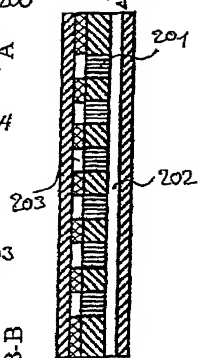


Fig. 5a

Fig. 5c



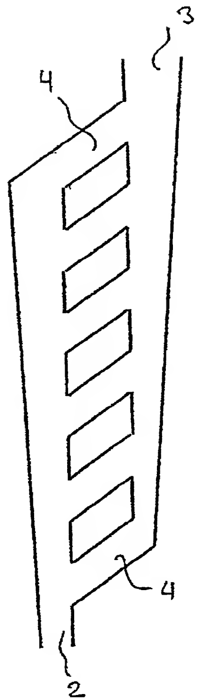


Fig. 6

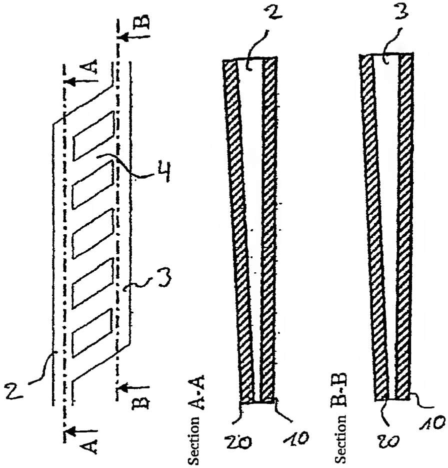


Fig. 7a Fig. 7b Fig. 7c

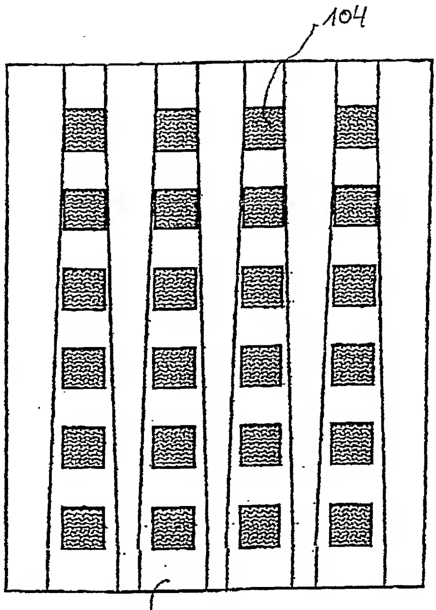


Fig. 8

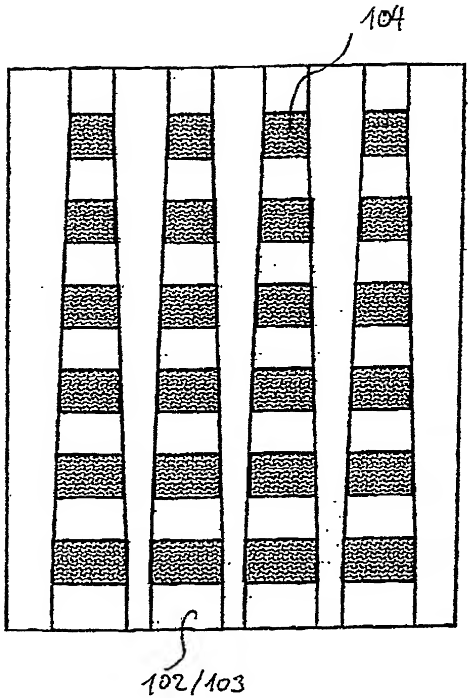


Fig. 9

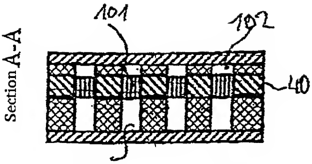


Fig. 10b

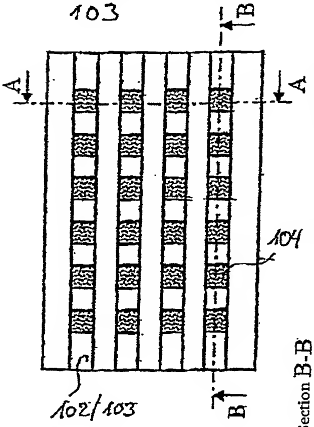


Fig. 10a

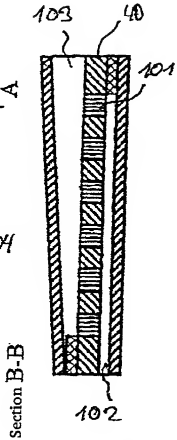


Fig. 10c

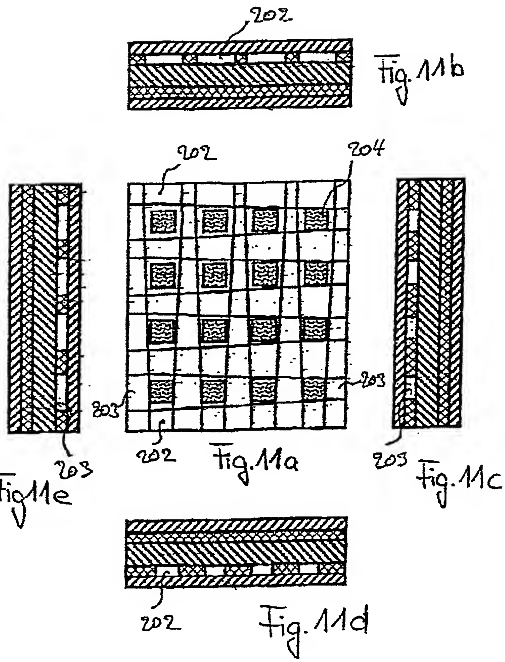


Fig. 11b

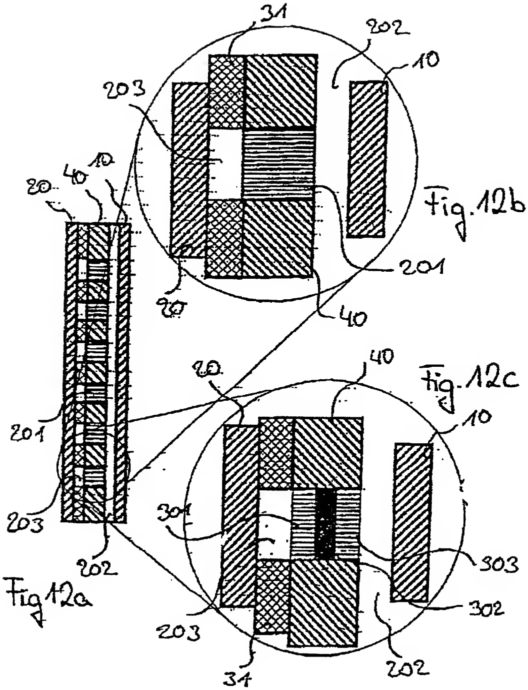
Fig. 11a

Fig. 11c

Fig 11e

Fig. 11d

10/030182


34
202
10
203
Fig.12b
201
20
40
20
40
10
201
203
202
Fig12a
20
40
Fig.12c
10
301
303
302
203
34
202

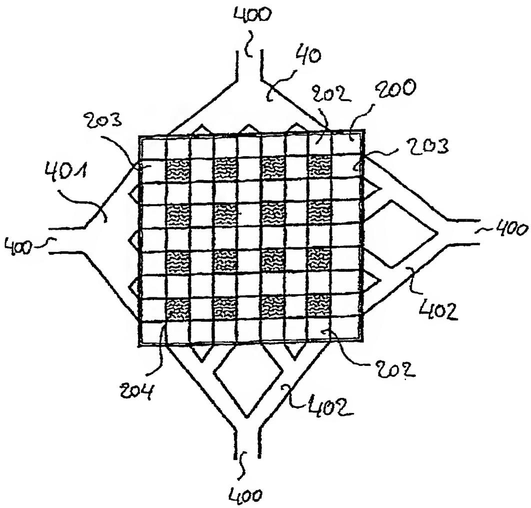


Fig. 13

10/030182

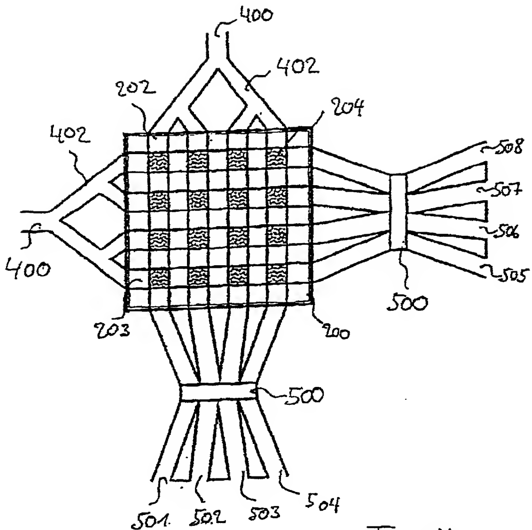


Fig:14

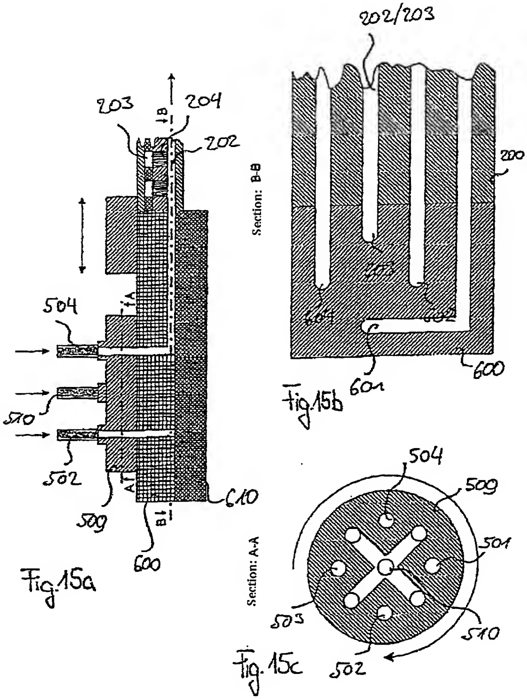


Fig.15a

Fig.15b

Fig.15c

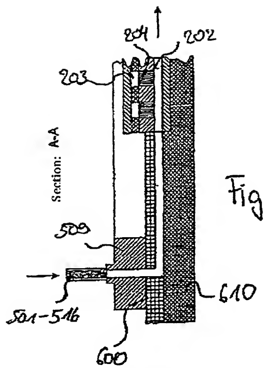


Fig. 16b

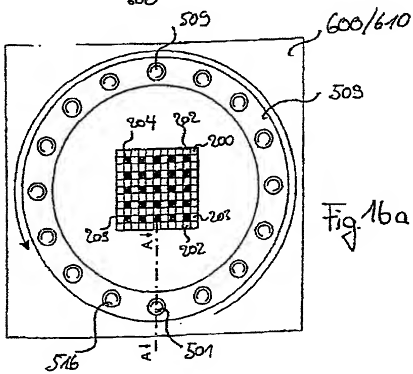


Fig. 16a.

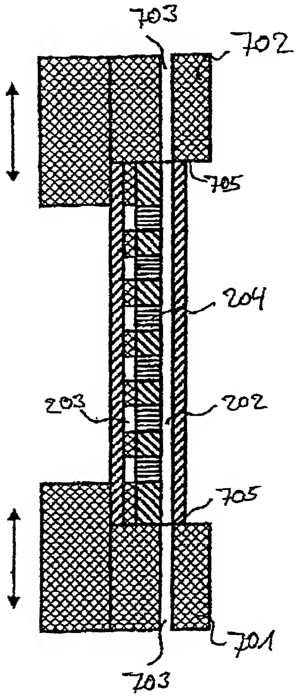


Fig.17

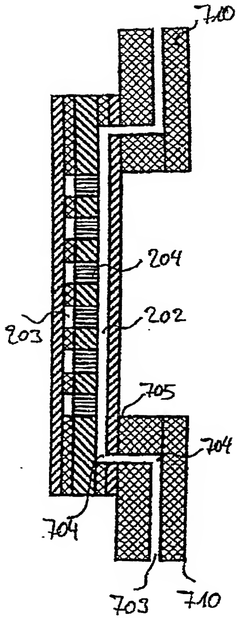

710
204
202
203
705
704
704
703
710

Fig. 18

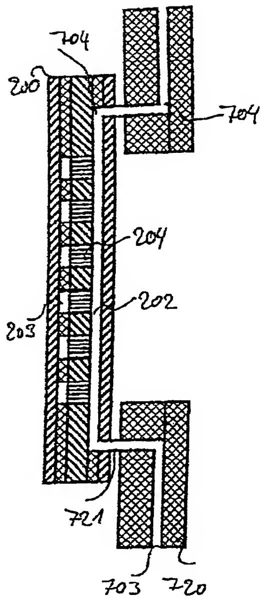

704
200
704
204
202
203
721
703
720

Fig. 19

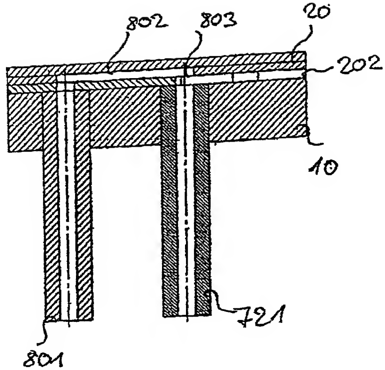


Fig. 20

21119P US-WO

Docket No. ____________

ARENT FOX KINTNER PLOTKIN & KAHN, PLLC
Nikaido, Marmelstein, Murray & Oram Intellectual Property Group

Declaration For U.S. Patent Application

As a below named inventor, I hereby declare that:
My residence, post office address and citizenship are as stated below my name.
I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled
(Insert Title) Microfluidic reaction support having three flow levels and a transparent cover layer
the specification of which is attached hereto unless the following box is checked:

☒ was filed on August 01, 2000 as PCT International Application Number PCT/EP00/07445 and was amended on Nov 03, 2000/Oct 25, 2001
and/or was filed on ____________ as United States Application Number ____________ and was amended on ____________.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claim(s), as amended by any amendment referred to above.
I acknowledge the duty to disclose information which is material to patentability as defined in 37 C.F.R. §1.56.
I hereby claim foreign priority benefits under 35 U.S.C. §119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below any foreign application for patent or inventor's certificate or PCT International Application having a filing date before that of the application(s) for which priority is claimed:

(List prior foreign applications. See note A on back of this page)	(Number)	(Country)	(Day/Month/Year Filed)	Priority Claimed
	199 35 433.2	DE	August 01, 1999	☒ Yes ☐ No
				☐ Yes ☐ No
				☐ Yes ☐ No

I hereby claim the benefit under 35 U.S.C. §119(c) of any United States provisional application(s) listed below.

(Application Number) (Filing Date)

(Application Number) (Filing Date)

(See Note B on back of this page) ☐ See attached list for additional prior foreign or provisional applications.

I hereby claim the benefit under 35 U.S.C. §120 of any United States application(s) or §365(c) of any PCT International application(s) designating the United States of America listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior application(s) (U.S. or PCT) in the manner provided by the first paragraph of 35, U.S.C. §112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 C.F.R. §1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

(List prior U.S. Applications or PCT International applications designating the U.S.)

(Application Serial No.) (Filing Date) (Status) (patented, pending, abandoned)

(Application Serial No.) (Filing Date) (Status) (patented, pending, abandoned)

And I hereby appoint as principal attorneys: Robert B. Murray, Reg. No. 22,980; Charles M. Marmelstein, Reg. No. 25,895; George E. Oram, Jr., Reg. No. 27,931; Douglas H. Goldhush, Reg. No. 33,125; David T. Nikaido, Reg. No. 22,663; Monica Chin Kitts, Reg. No. 36,105; Richard J. Berman, Reg. No. 39,107; King L. Wong, Reg. No. 37,500; James A. Poulos, III, Reg. No. 31,714; Patrick D. Muir, Reg. No. 37,403; Murat Ozgu, Reg. No. 44,275; Bradley D. Goldizen, Reg. No. 43,637, N. Alexander Nolte, Reg. No. 45,689 and Robert K. Carpenter, Reg. No. 34,794. 14

Please direct all communications to the following address: ARENT FOX KINTNER PLOTKIN & KAHN, PLLC
1050 Connecticut Avenue, N.W., Suite 600
Washington, D.C. 20036-5339
Telephone No. (202) 857-6000; Facsimile No. (202) 638-4810

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

(See Note C on back of this page)

1-00
Full name of sole or first inventor STÄHLER Cord Friedrich
Inventor's signature X [signature] Date x06.02.02
Residence ~~Siegfriedstraße 9~~, 69469 Weinheim, Germany DEX
Citizenship German Gaesselweg 15
Post Office Address same as above CFS 6.2.2002

Full name of second joint inventor, if any 2-00 MÜLLER Manfred

Inventor's signature x [signature] x 06.02.02 Date

Residence Reutterstraße 76b, 80689 München, Germany DEX

Citizenship German

Post Office Address same as above

Full name of third joint inventor, if any 3-00 STÄHLER Peer Friedrich

Inventor's signature x [signature] x 06.02.02 Date

Residence Riedfeldstraße 3, 68169 Mannheim, Germany DEX

Citizenship German

Post Office Address same as above

Full name of fourth joint inventor, if any

Inventor's signature Date

Residence

Citizenship

Post Office Address

Full name of fifth joint inventor, if any

Inventor's signature Date

Residence

Citizenship

Post Office Address

Full name of sixth joint inventor, if any

Inventor's signature Date

Residence

Citizenship

Post Office Address

Full name of seventh joint inventor, if any

Inventor's signature Date

Residence

Citizenship

Post Office Address

Full name of eighth joint inventor, if any

Inventor's signature Date

Residence

Citizenship

Post Office Address

Full name of ninth joint inventor, if any

Inventor's signature Date

Residence

Citizenship

Post Office Address